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Cloning immunoglobulin variable domains for expression by the polymerase chain reaction

(chimeric antibodies/MBr1)

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cDNA Synthesis and Amplification. RNA was prepared from about 5×10^8 hybridoma cells grown in roller bottles, and mRNA was selected on oligo(dT)-cellulose (25). First-strand cDNA synthesis was based on ref. 26. A 50-μl reaction mixture containing 10 μg of mRNA, 20 pmol of VH1FOR primer [5'-d(TGAGGAGACGGTGACCGTGGTCCCTTGG-CCCCAG)] or VK1FOR primer [5'-d(GTTAGATCTCCAG-CTTGGTCCC)], 250 μM of each dNTP, 10 mM dithiothreitol, 100 mM Tris·HCl (pH 8.3), 10 mM $MgCl_2$, and 140 mM KCl was heated at 70°C for 10 min and cooled. Reverse transcriptase (Anglian Biotec, Colchester, U.K.) was added (46 units) and incubated at 42°C for 1 hr. For amplification with a thermostable DNA polymerase (15), a 50-μl reaction mixture containing 5 μl of the cDNA·RNA hybrid, 25 pmol of primers VH1FOR or VK1FOR and VH1BACK [5'-d(AGGTSMARC-TGCAGSAGTCWGG) in which S = C or G, M = A or C, R = A or G, and W = A or T] or VK1BACK [5'-d(GA-CATTCAGCTGACCCAGTCTCCA)] as appropriate, 250 μM of each dNTP, 67 mM Tris chloride (pH 8.8), 17 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 200 μg of gelatine per ml, and 2 units of thermus aquaticus (Taq) polymerase (Cetus) was overlaid with paraffin oil and subjected to 25 rounds of temperature cycling with a Techne PHC-1 programable heating block. A typical cycle was 1 min at 95°C (denature), 1 min at 30°C (anneal), and 2 min at 72°C (elongate). The sample (and oil) was extracted twice with ether, once with phenol, and then with phenol/$CHCl_3$, followed by ethanol precipitation. The sample was taken up in 50 μl of water and frozen.

PCR

Table 1. Checking primers for mismatches with the data base entries

Primers	Entries with none, one, or two mismatches		
	0	1	2
VH1FOR	50/131	71/131	
VH1BACK	22/141	56/141	43/141
VK1FOR	38/61	20/61	
VK1BACK	19/115	54/115	26/115

For each primer, the number of entries with zero, one, or two mismatches with respect to the 14 nucleotides at the 3' end of each of the primers are given, as well as the total number of eligible entries. For the mixed VH1BACK primer, each of the possible variants was scored and the results summed.

Here's one AA intergene at one end & the other at the other

110 AA ⇒ 330 bases

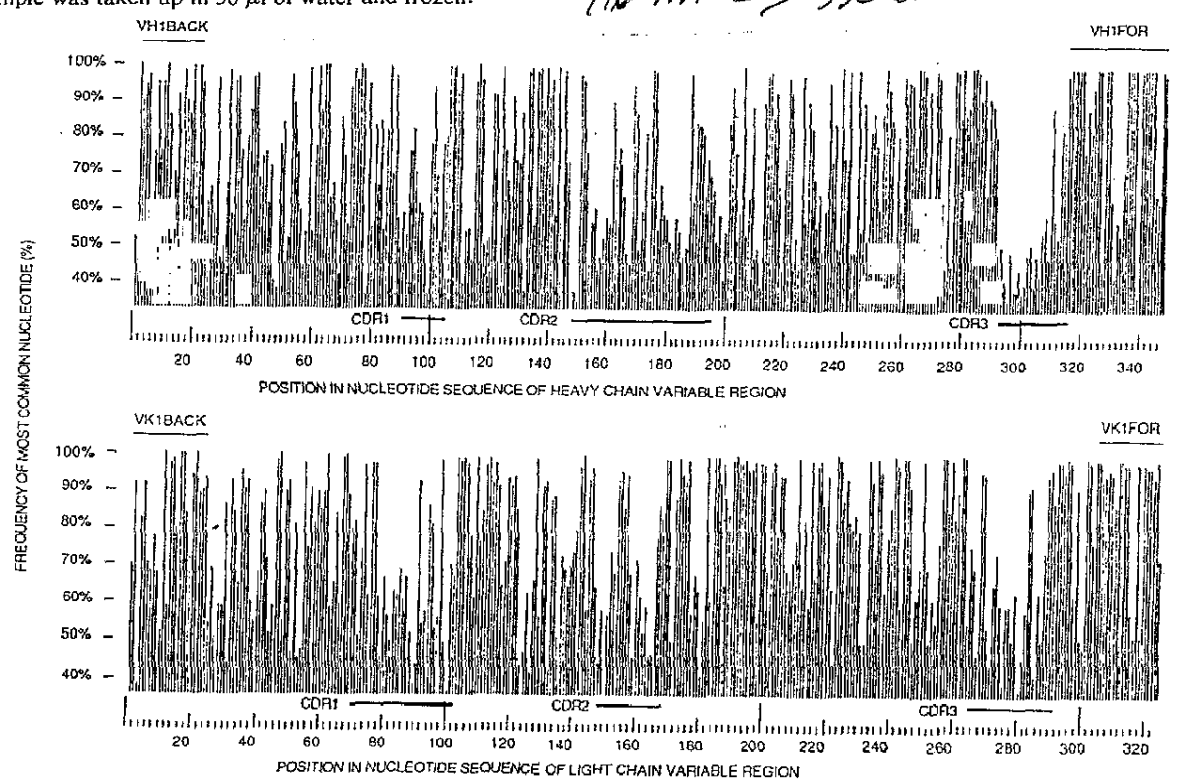


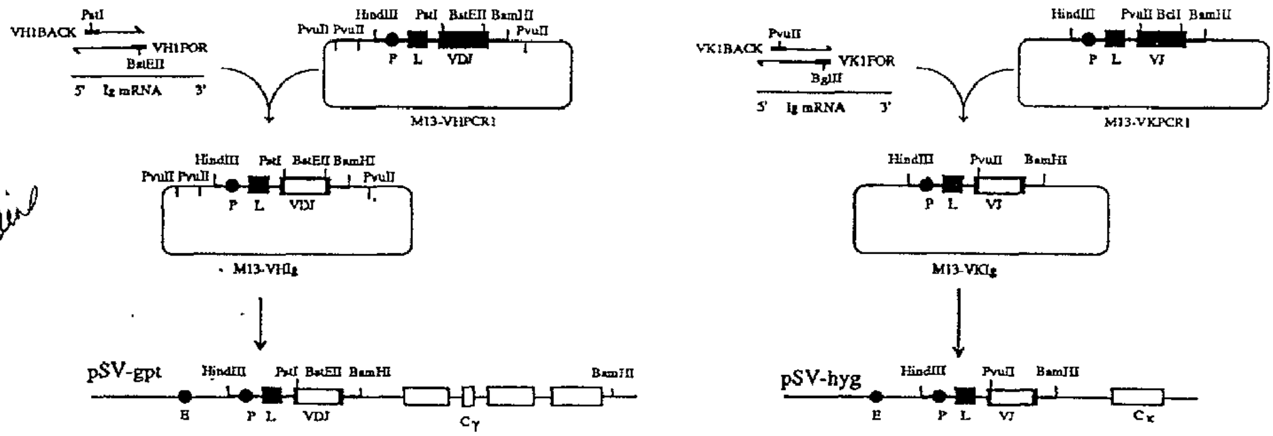
FIG. 1. Frequency of the most common nucleotides in V_H and V_κ gene sequences in ref. 23. CDR1, CDR2, and CDR3 are located,

PM3001193190

Given primer sequence would probably be able to prime seqs of many mouse V region — Seq of this work

light chain

Heavy chain



PstI
VH1BACK
VH1FOR
BstEII
5' Ig mRNA 3'
HindIII PstI BstEII BamHI
PvuII PvuII
PvuII
P L VDJ
M13-VHPCR1
PvuII PvuII HindIII PstI BstEII BamHI PvuII
P L VDJ
M13-VHIg
pSV-gpt
HindIII PstI BstEII BamHI
BamHI
E P L VDJ Cγ
PvuII
VK1BACK
VK1FOR
BglII
5' Ig mRNA 3'
HindIII PvuII BclI BamHI
P L VJ
M13-VKPCR1
HindIII PvuII BamHI
P L VJ
M13-VKIg
pSV-hyg
HindIII PvuII BamHI
E P L VJ Cκ

FIG. 2. Scheme for amplification of cDNA and cloning into phage M13 vectors to hook up V region genes for expression. The vectors M13-VHPCR1 and M13-VKPCR1, for cloning the amplified cDNA, contain introns: transcription is driven from the immunoglobulin heavy chain promoter (P), and the signal sequence (L) and leader intron are taken from the mouse V47 unrearranged V_H gene (12). The noncoding sequence to the 3' end of the V_H gene is described in ref. 12 and of the V_κ gene in ref. 9.

Bank made by PCR + directly into expression vector

IA	.glu.val.gln.leu.gln.glu.ser.gly.pro.ser
IB	.gln.val.gln.leu.lys.glu.ser.gly.pro.gly
IIA	.glu.val.gln.leu.gln.gln.ser.gly.pro.glu
IIB	.gln.val.gln.leu.gln.gln.pro.gly.ala.glu
IIC	.glu.val.gln.leu.gln.gln.ser.gly.ala.glu
IIIA	.glu.val.lys.leu.val.glu.ser.gly.gly.gly
IIIB	.glu.val.lys.leu.leu.glu.ser.gly.gly.gly
IIIC	.glu.val.lys.leu.glu.glu.ser.gly.gly.gly
IIID	.glu.val.gln.leu.val.glu.ser.gly.gly.gly
VA	.glu.val.gln.leu.gln.gln.ser.gly.ala.glu
VB	.glu.val.gln.leu.gln.gln.ser.gly.ala.glu

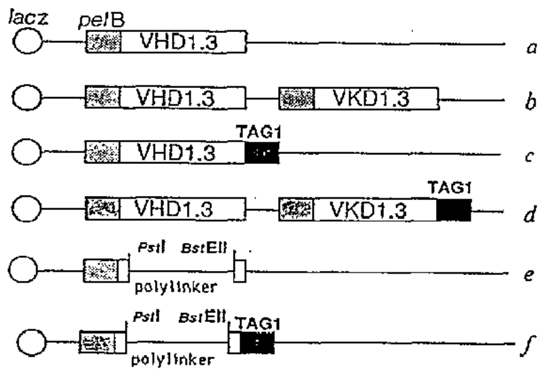
AG.GT^C_G.^{A}A$_C^A$.CTG.CAG.^C_GAG.TC^A_T.GG

glu.val.gln.leu.gln.glu.ser.glu
gln lys gln

Pst

Bull tried to repeat this & had some trouble. "Word going round" is that some Ab are easier than other

another article expression by E. coli



g

HindIII
pUC19..........AAGCTTGCATG

pelB
M K Y L L P T A A
CAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC
pelB ↓
A G L L L L A A Q P A M A Q V Q L Q
GCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAG
PstI

Polylinker
TCTAGAGTCGACCTCGAG
XbaI SalI XhoI

V T V S S E Q K L I S E E D L N * *
GGTCACCGTCTCCTCAGAACAAAAACTCATCTCAGAAGAGGATCTGAATTAATAA
BstEII

GGGCTAAGCTCGAATTC.......pUC19
EcoRI